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DATA EVALUATION RECORD

I. SUMMARY

MRID (Acc.) No.: 412552-23
ID No.: 7078-RT
RD Record No.: 253,112
Caswell No.: 623C (129017)
Project No.: 0-0339

Study Type: Mutagenicity - Gene mutation in mammalian cells in vitro (CHO/HGPRT)

Chemical: CIDEX OPA Antimicrobial (o-phthalaldehyde)

Sponsor: Surgikos, Inc., Arlington, TX

Testing Facility: Microbiological Associates (M/A)
Bethesda, MD

Title of Report: CHO/HGPRT Mutation Assay, with Confirmation.

Authors: John H. Harbell

Study Number: (M/A) T8241.332001

Date of Issue: December 28, 1988

TB Conclusions:

Negative for inducing forward mutation at the HGPRT locus of CHO cells exposed in vitro with/without metabolic activation to test article up to cytotoxic levels (20 ug/mL -S9; 30 ug/mL +S9).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - 913-12 (ortho-phthalaldehyde, OPA)

Description: Light yellow crystalline solid
Batch (Lot): 861-65
Purity (%): 99.7
Solvent/Carrier/Diluent: Distilled water (DW)

B. Test Organism - Mammalian cell culture strain

Species: Chinese hamster (ovary, CHO)
Strain: K_1-BH_4
Source: Dr. Abraham Hsie, Oak Ridge National Lab (TN)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of OPA when administered in vitro to CHO cells, according to a submitted protocol based upon validated published procedures.

A Statement of Quality Assurance measures (inspections/audits) was provided, as well as a statement of adherence to Good Laboratory Practice.

D. Procedures/Methods of Analysis - Following preliminary toxicity testing (9 concentrations of test article ranging from 0.1 through 1000 ug/mL), triplicate cultures of CHO cells were exposed to five dose levels of OPA for 5 hours, both in the absence and presence of a mammalian metabolic activation system consisting of the microsomal (S9) fraction of liver homogenates from male Fischer 344 rats pretreated with Aroclor-1254, plus NADP(H)-generating cofactors. In addition to solvent controls (DW), other cultures were exposed to ethyl methanesulfonate (EMS, 0.2 ul/mL) or to benzo(a)pyrene (BaP, 4 ug/mL) as positive controls for the nonactivated and activated series, respectively.

Following treatment, all dish cultures were washed free of test substances, and incubated in fresh medium for a further 18 to 24 hours, following which they were subcultured for up to 7 to 9 days, in order to express phenotype (expression period). Selection for the mutant (TG-resistant phenotypes) was then performed in media containing 10 uM thioguanine (TG), allowing up to 10 days of further incubation to assess mutant selection. After this period (minimum of 7 days), cultures were fixed, stained, and prepared for microscopic examination.

For the calculation of mutagenic response, the numbers of mutant clones in treated cultures are compared to background mutant frequency by a one-sided Student

t-test, such values considered significant when: 1) Treatment frequencies are increased above that of either solvent or untreated controls by at least 10.8 mutants per 10^6 clonable cells (the confidence interval employed by this lab for the CHO/HGPRT assay); and 2) test frequencies are at least twice that of negative controls. Thus, a test article is registered as positive when the value in test cultures exceeds at least 20 mutants per 10^6 clonable cells. An assay would be considered positive when (according to the investigator):

> ". . . a dose-dependent increase in mutant frequency was observed and one or more of the five concentrations tested induced a significant increase in mutant frequency. A significant increase would be one which was 1) greater than 20 mutants per 10^6 clonable cells, 2) at least twice that of the solvent control and untreated control, and 3) increased above that of the solvent control and the untreated control by at least 10.8 mutants per 10^6 clonable cells. An assay would be considered suspect if there was no dose response but one or more doses induced a significant increase in the mutant frequency. The assay would be considered negative if none of the doses tested induced a mutant frequency which was considered significant."

In addition, this lab considers assays valid for evaluation only when:

1. The cloning efficiency of negative controls must be 50 percent or greater.

2. The spontaneous (background) mutant frequency in negative controls falls in the range of 0 to 20 mutants/10^6 cells.

3. The positive control value is at least three times that of the solvent control value.

E. Results - Doses selected for the mutagenicity assays from the preliminary toxicity testing were 20, 12, 10, 5, and 1 ug/mL in the absence of activation (-S9); 30, 23, 16, 9, and 3 ug/mL in its presence (+S9).

These doses were based on the determination of cloning efficiencies (CE) in test cultures relative to solvent controls which showed nonactivated doses of 30 ug/mL

and above as well as activated doses of 100 ug/mL and above were excessively toxic (respectively, 0.03% CE and 0% CE); lower concentrations gave respectable dose-related CE approaching background (Report Table 1).

Determination of cytotoxicity concurrently with the first mutagenicity assay was comparable to that found in dose-selection tests (Report Table 2, attached to this DER), but at no concentration of OPA did mutant frequencies exceed background ($20/10^6$), with or without activation, nor significantly increased above solvent control (Report Tables 3 and 4, attached).

In an independent repeat (confirmatory) assay at the same test dose schedules, none of the cultures treated at levels of OPA that could be cloned (higher doses were too toxic for a mutagenic assessment) were significantly above concurrent solvent controls, nor exceeded historical background (Report Tables 5, 6, and 7, attached).

By contrast in both assays, EMS and BaP positive controls responded appropriately, with mutant frequencies well above control values (Tables 3, 4, 6, and 7).

The author concluded that, under conditions of this assay, OPA was negative for induction of gene mutation at the HGPRT locus of CHO cells.

F. TB Evaluation - ACCEPTABLE. This study was conducted under adequate conditions and appropriate control procedures such that the data generated support the author's conclusion of a negative result.

Attachments (Data Tables)

ATTACHMENT I

Report Data Tables

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